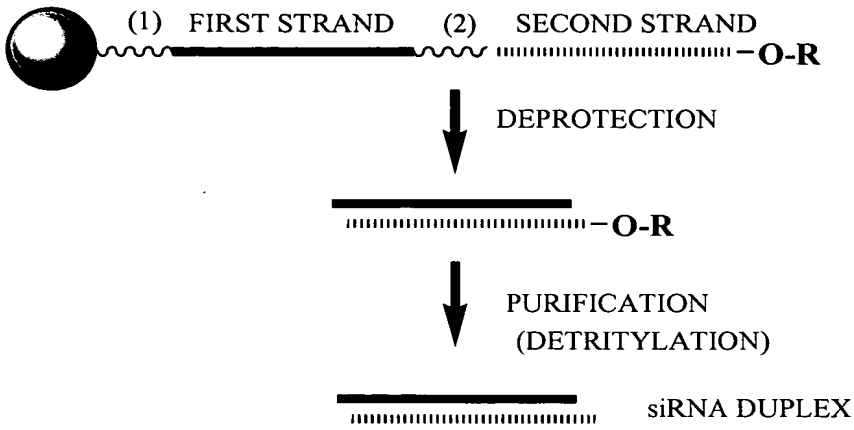
Figure 1

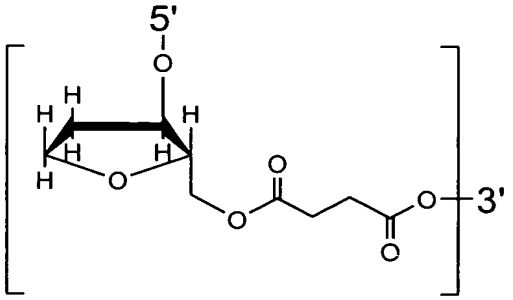


= SOLID SUPPORT

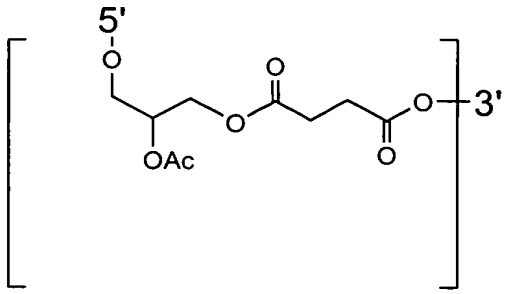
R = TERMINAL PROTECTING GROUP
FOR EXAMPLE:
DIMETHOXYTRITYL (DMT)

(1) = CLEAVABLE LINKER
(FOR EXAMPLE: NUCLEOTIDE SUCCINATE OR INVERTED DEOXYABASIC SUCCINATE)

(2) = CLEAVABLE LINKER
(FOR EXAMPLE: NUCLEOTIDE SUCCINATE OR INVERTED DEOXYABASIC SUCCINATE)



INVERTED DEOXYABASIC SUCCINATE LINKAGE



GLYCERYL SUCCINATE LINKAGE

Figure 2

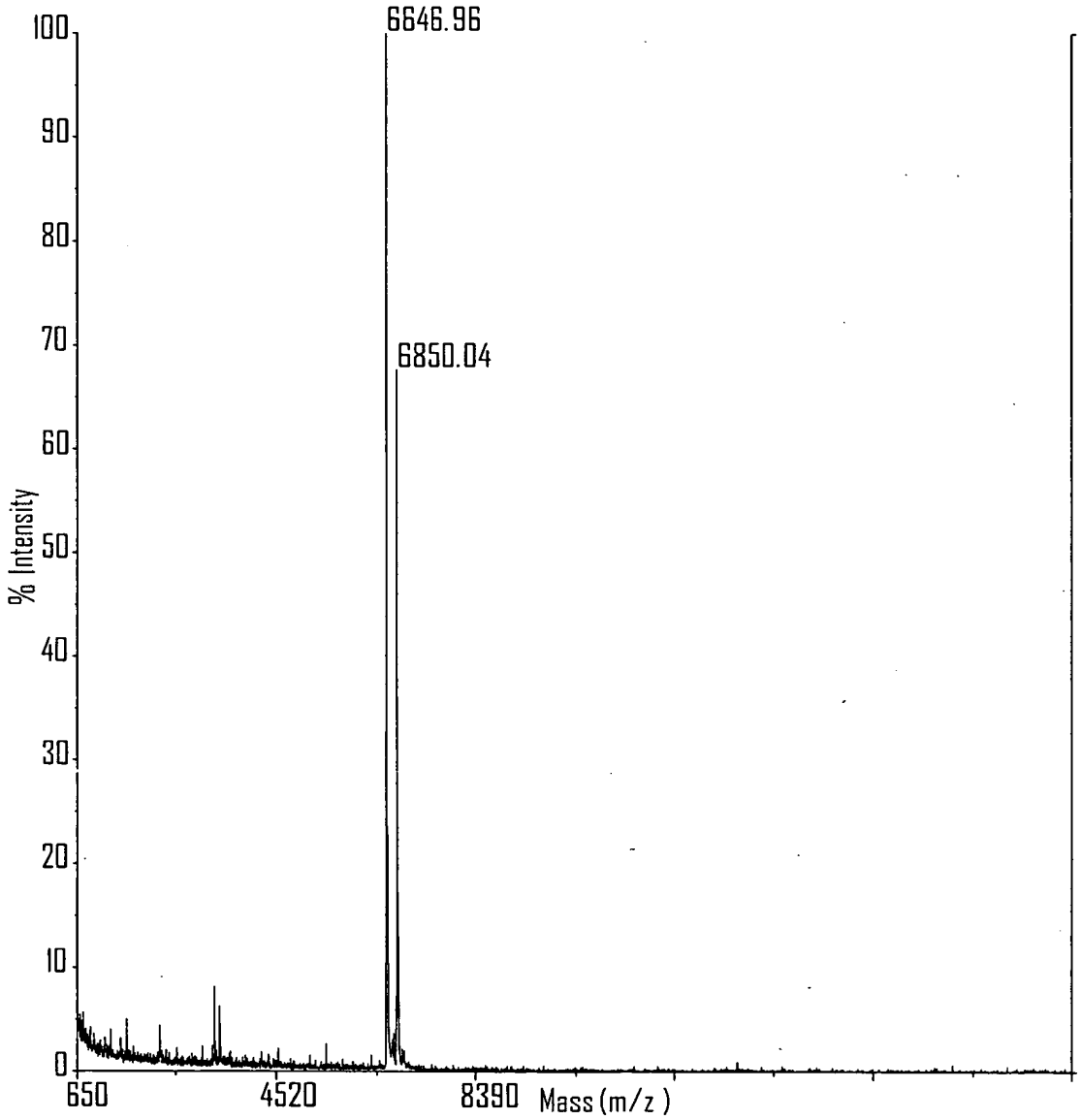

6646.96
6850.04
100
90
80
70
60
50
40
30
20
10
0
% Intensity
650
4520
8390
Mass (m/z)

Figure 3

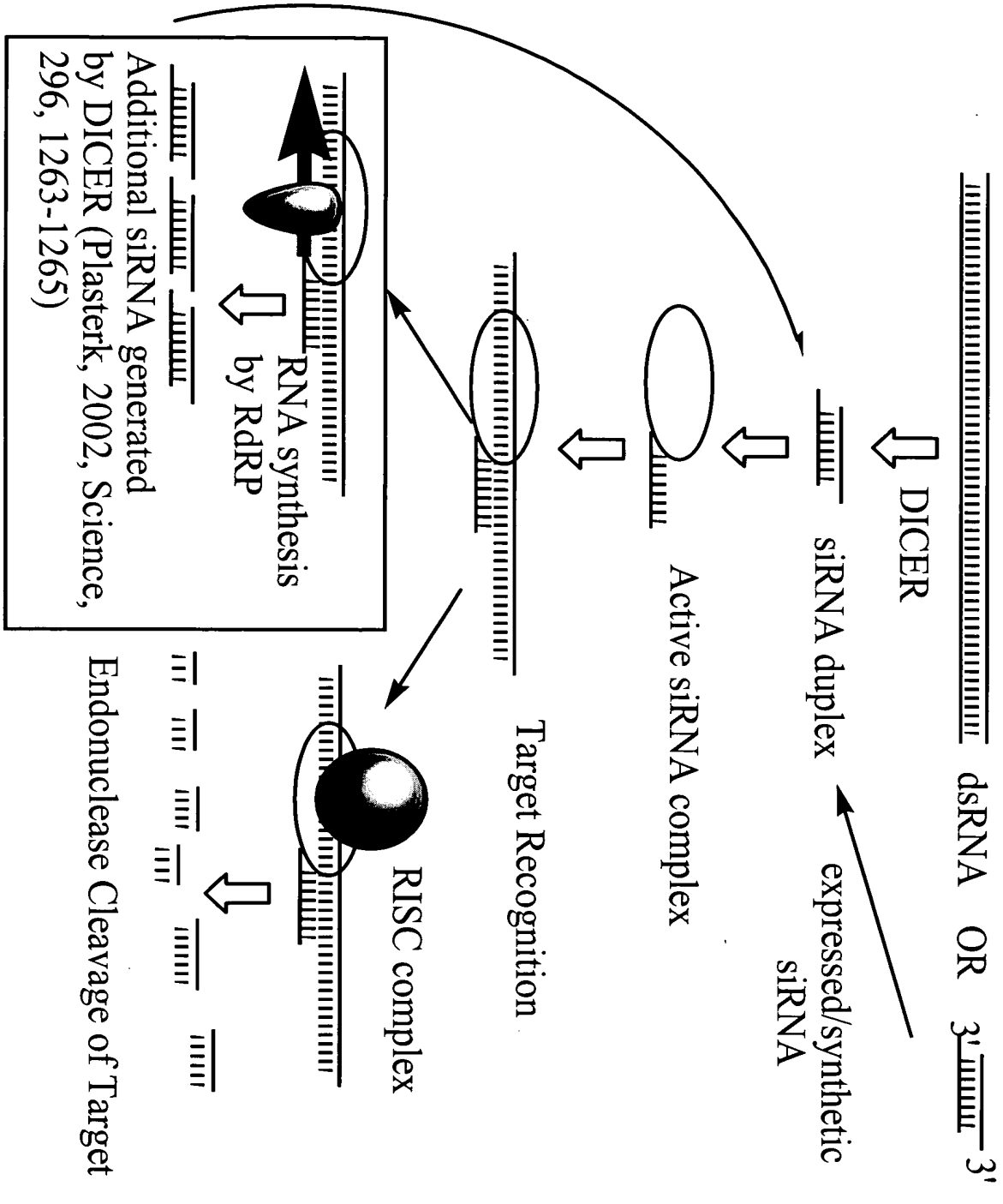

dsRNA OR 3'
DICER
siRNA duplex
expressed/synthetic siRNA
Active siRNA complex
Target Recognition
RISC complex
RNA synthesis by RdRP
Additional siRNA generated by DICER (Plasterk, 2002, Science, 296, 1263-1265)
Endonuclease Cleavage of Target

Figure 4

A

SENSE STRAND (SEQ ID NO 456)
ALL POSITIONS RIBONUCLEOTIDE EXCEPT POSITIONS (N N)

5'- B-N N N N N N N N N N N N N N N N N N N (N N)-B -3'
3'- L-(N_SN) N N N N N N N N N N N N N N N N N N N -5'

ANTISENSE STRAND (SEQ ID NO 457)
ALL POSITIONS RIBONUCLEOTIDE EXCEPT POSITIONS (N N)

B

SENSE STRAND (SEQ ID NO 458)
ALL PYRIMIDINES = 2'-FLUORO AND ALL PURINES = 2'-OM EXCEPT POSITIONS (N N)

5'- N N N N N N N N N N N N N N N N N N N (N_SN) -3'
3'- L-(N_SN) N N N N N N N N N N N N N N N N N N N -5'

ANTISENSE STRAND (SEQ ID NO 459)
ALL PYRIMIDINES = 2'-FLUORO AND ALL PURINES = 2'-O-ME EXCEPT POSITIONS (N N)

C

SENSE STRAND (SEQ ID NO 460)
ALL PYRIMIDINES = 2'-O-ME OR 2'-FLUORO EXCEPT POSITIONS (N N)

5'- B-N N N N N N N N N N N N N N N N N N N (N N)-B -3'
3'- L-(N_SN) N N N N N N N N N N N N N N N N N N N -5'

ANTISENSE STRAND (SEQ ID NO 461)
ALL PYRIMIDINES = 2'-FLUORO EXCEPT POSITIONS (N N)

D

SENSE STRAND (SEQ ID NO 462)
ALL PYRIMIDINES = 2'-FLUORO EXCEPT POSITIONS (N N) AND ALL PURINES = 2'-DEOXY

5'- B-N N N N N N N N N N N N N N N N N N N (N N)-B -3'
3'- L-(N_SN) N N N N N N N N N N N N N N N N N N N -5'

ANTISENSE STRAND (SEQ ID NO 459)
ALL PYRIMIDINES = 2'-FLUORO AND ALL PURINES = 2'-O-ME EXCEPT POSITIONS (N N)

E

SENSE STRAND (SEQ ID NO 463)
ALL PYRIMIDINES = 2'-FLUORO EXCEPT POSITIONS (N N)

5'- B-N N N N N N N N N N N N N N N N N N N (N N)-B -3'
3'- L-(N_SN) N N N N N N N N N N N N N N N N N N N -5'

ANTISENSE STRAND (SEQ ID NO 459)
ALL PYRIMIDINES = 2'-FLUORO AND ALL PURINES = 2'-O-ME EXCEPT POSITIONS (N N)

F

SENSE STRAND (SEQ ID NO 462)
ALL PYRIMIDINES = 2'-FLUORO EXCEPT POSITIONS (N N) AND ALL PURINES = 2'-DEOXY

5'- B-N N N N N N N N N N N N N N N N N N N (N N)-B -3'
3'- L-(N_SN) N N N N N N N N N N N N N N N N N N N -5'

ANTISENSE STRAND (SEQ ID NO 464)
ALL PYRIMIDINES = 2'-FLUORO EXCEPT POSITIONS (N N) AND ALL PURINES = 2'-DEOXY

POSITIONS (NN) CAN COMPRISE ANY NUCLEOTIDE, SUCH AS DEOXYNUCLEOTIDES (eg. THYMIDINE) OR UNIVERSAL BASES
B = ABASIC, INVERTED ABASIC, INVERTED NUCLEOTIDE OR OTHER TERMINAL CAP THAT IS OPTIONALLY PRESENT
L = GLYCERYL or B THAT IS OPTIONALLY PRESENT
S = PHOSPHOROTHIOATE OR PHOSPHORODITHIOATE that is optionally absent

Figure 5

A

SENSE STRAND (SEQ ID NO 465)

5'- iB- U G U C U A U C A G C G C A G C U A C *T T*-iB -3'

3'- L-T_S*T* A C A G A U A G U C G C G U C G A U G -5'

ANTISENSE STRAND (SEQ ID NO 466)

B

SENSE STRAND (SEQ ID NO 467)

5'- *u* g *u c u* a *u c* a g *c* g *c* a g *c u* a *c* T_S*T* -3'

3'- L-T_S*T* a c a g a *u* a g *u c* g *c* g *u c* g a *u* g -5'

ANTISENSE STRAND (SEQ ID NO 468)

C

SENSE STRAND (SEQ ID NO 469)

5'- iB-u G u c u A u c A G c G c A G c u A c *T T*-iB -3'

3'- L-T_S*T* A *c* A G A *u* A G *u c* G *c* G *u c* G A *u* G -5'

ANTISENSE STRAND (SEQ ID NO 470)

D

SENSE STRAND (SEQ ID NO 471)

5'- iB-*u G u c u A u c A G c G c A G c u A c T T*-iB -3'

3'- L-T_S*T* a c a g a *u* a g *u c* g *c* g *u c* g a *u* g -5'

ANTISENSE STRAND (SEQ ID NO 468)

E

SENSE STRAND (SEQ ID NO 472)

5'- iB-*u* G *u c u* A *u c* A G *c* G *c* A G *c u* A *c T T*-iB -3'

3'- L-T_S*T* a c a g a *u* a g *u c* g *c* g *u c* g a *u* g -5'

ANTISENSE STRAND (SEQ ID NO 468)

F

SENSE STRAND (SEQ ID NO 471)

5'- iB-*u G u c u A u c A G c G c A G c u A c T T*-iB -3'

3'- L-T_S*T A c A G A u A G u c G c G u c G A u G* -5'

ANTISENSE STRAND (SEQ ID NO 473)

lower case = 2'-O-Methyl or 2'-deoxy-2'-fluoro

italic lower case = 2'-deoxy-2'-fluoro

underline = 2'-O-methyl

ITALIC UPPER CASE = DEOXY

iB = INVERTED DEOXYABASIC

L = GLYCERYL MOIETY or iB OPTIONALLY PRESENT

S = PHOSPHOROTHIOATE OR PHOSPHORODITHIOATE OPTIONALLY PRESENT

Figure 6

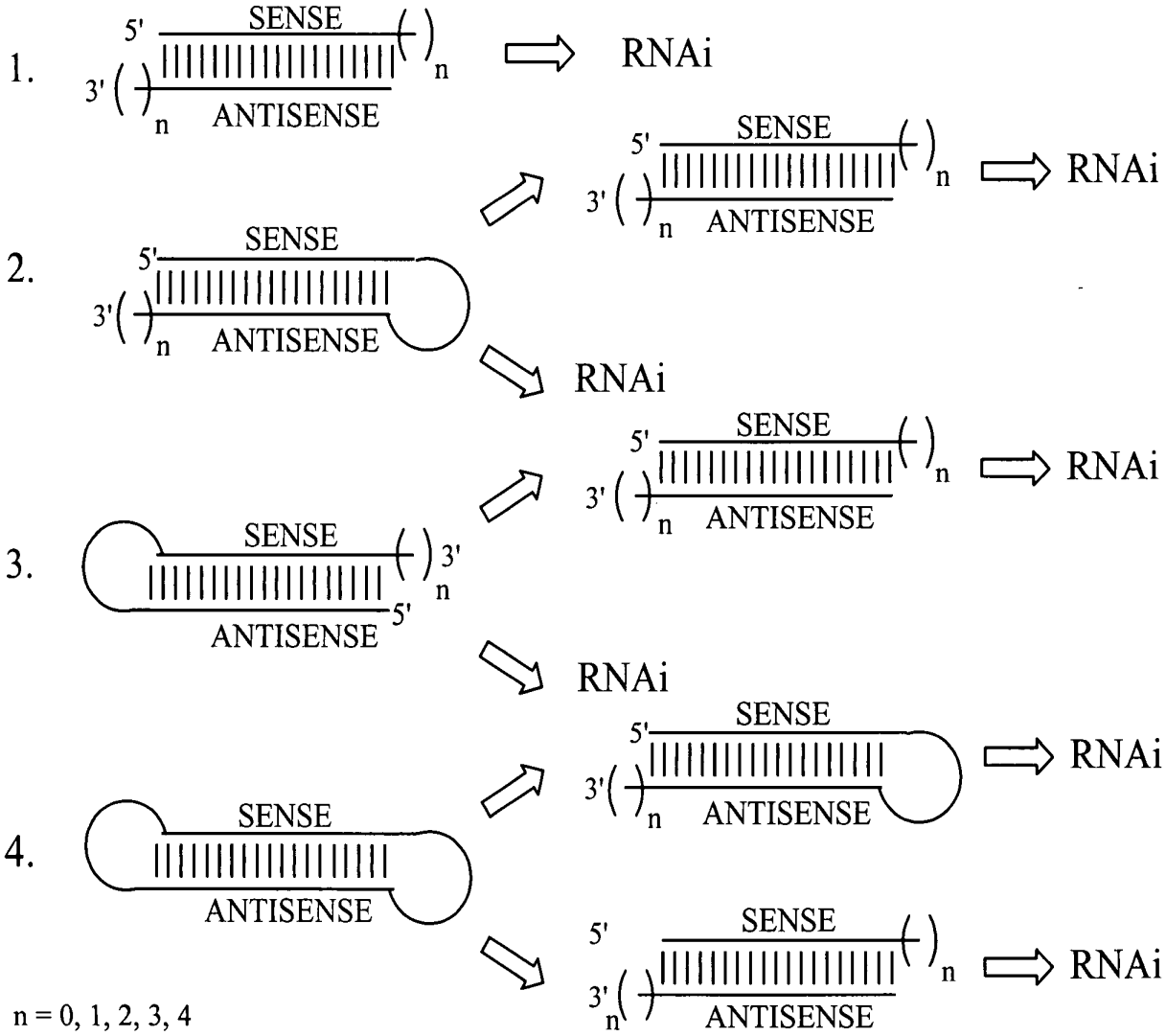
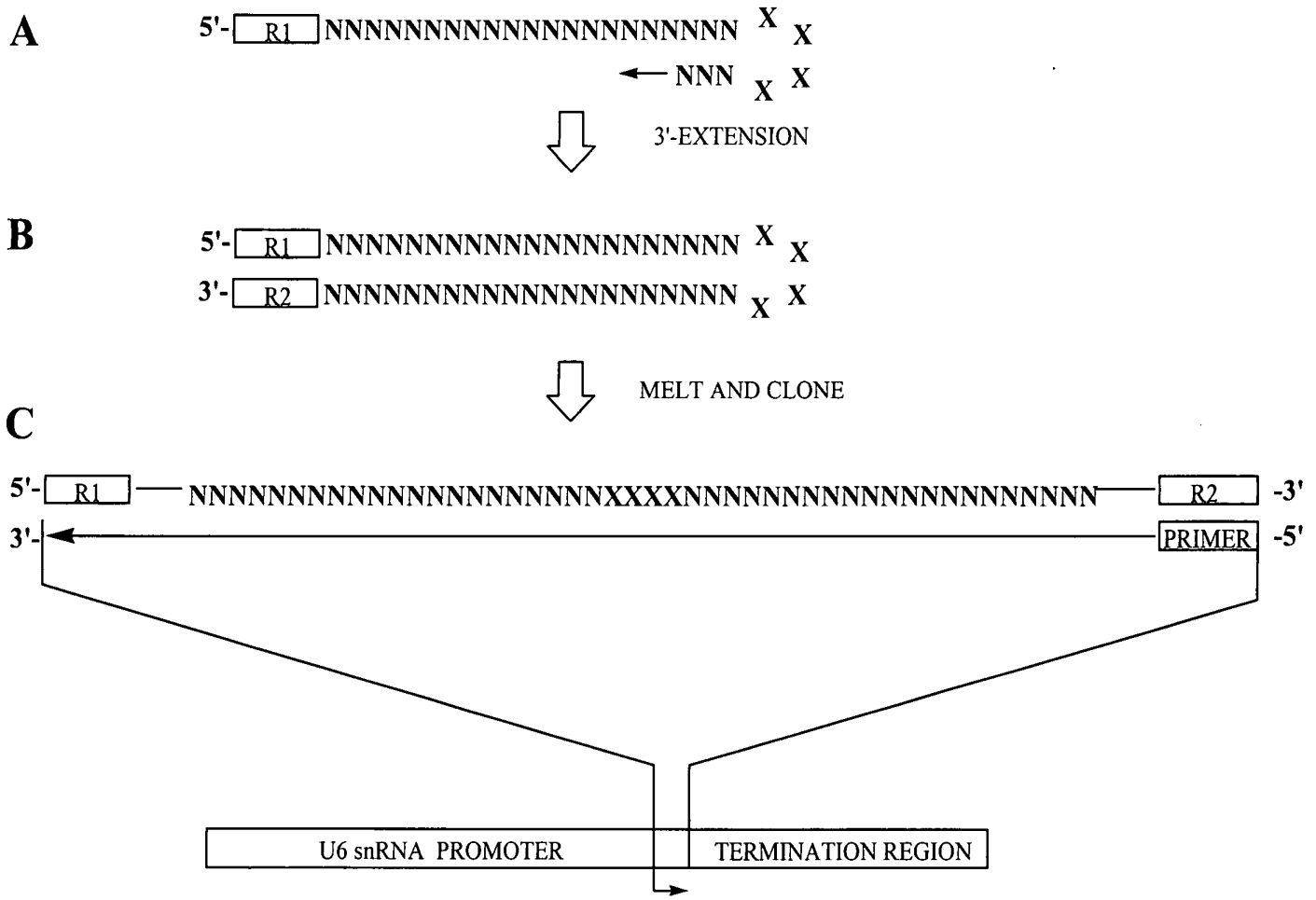

1.
5'
SENSE
3'
n
ANTISENSE
RNAi
2.
5'
SENSE
3'
n
ANTISENSE
RNAi
3.
SENSE
3'
n
5'
ANTISENSE
RNAi
4.
SENSE
ANTISENSE
RNAi
n = 0, 1, 2, 3, 4

Figure 7



R1 = RESTRICTION SITE #1
R2 = RESTRICTION SITE #2
N = A, G, C, or T
X = A, G, C, or T LOOP SEQUENCE

Figure 8

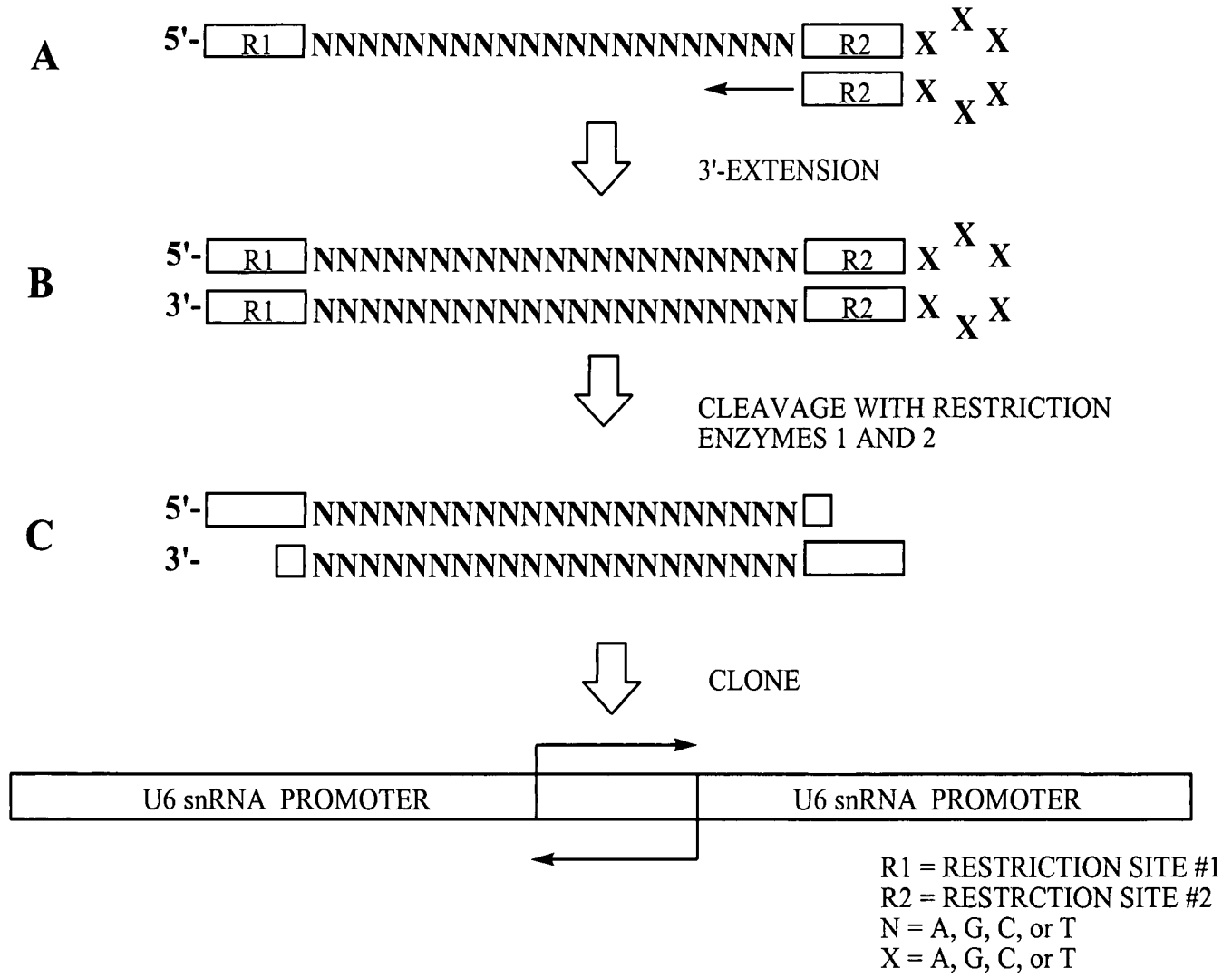

A
5'- R1 NNNNNNNNNNNNNNNNNNN R2 X X X
R2 X X X
3'-EXTENSION
B
5'- R1 NNNNNNNNNNNNNNNNNNN R2 X X X
3'- R1 NNNNNNNNNNNNNNNNNNN R2 X X X
CLEAVAGE WITH RESTRICTION ENZYMES 1 AND 2
C
5'- NNNNNNNNNNNNNNNNNNN
3'- NNNNNNNNNNNNNNNNNNN
CLONE
U6 snRNA PROMOTER
U6 snRNA PROMOTER
R1 = RESTRICTION SITE #1
R2 = RESTRCTION SITE #2
N = A, G, C, or T
X = A, G, C, or T

Figure 9: Target site Selection using siRNA

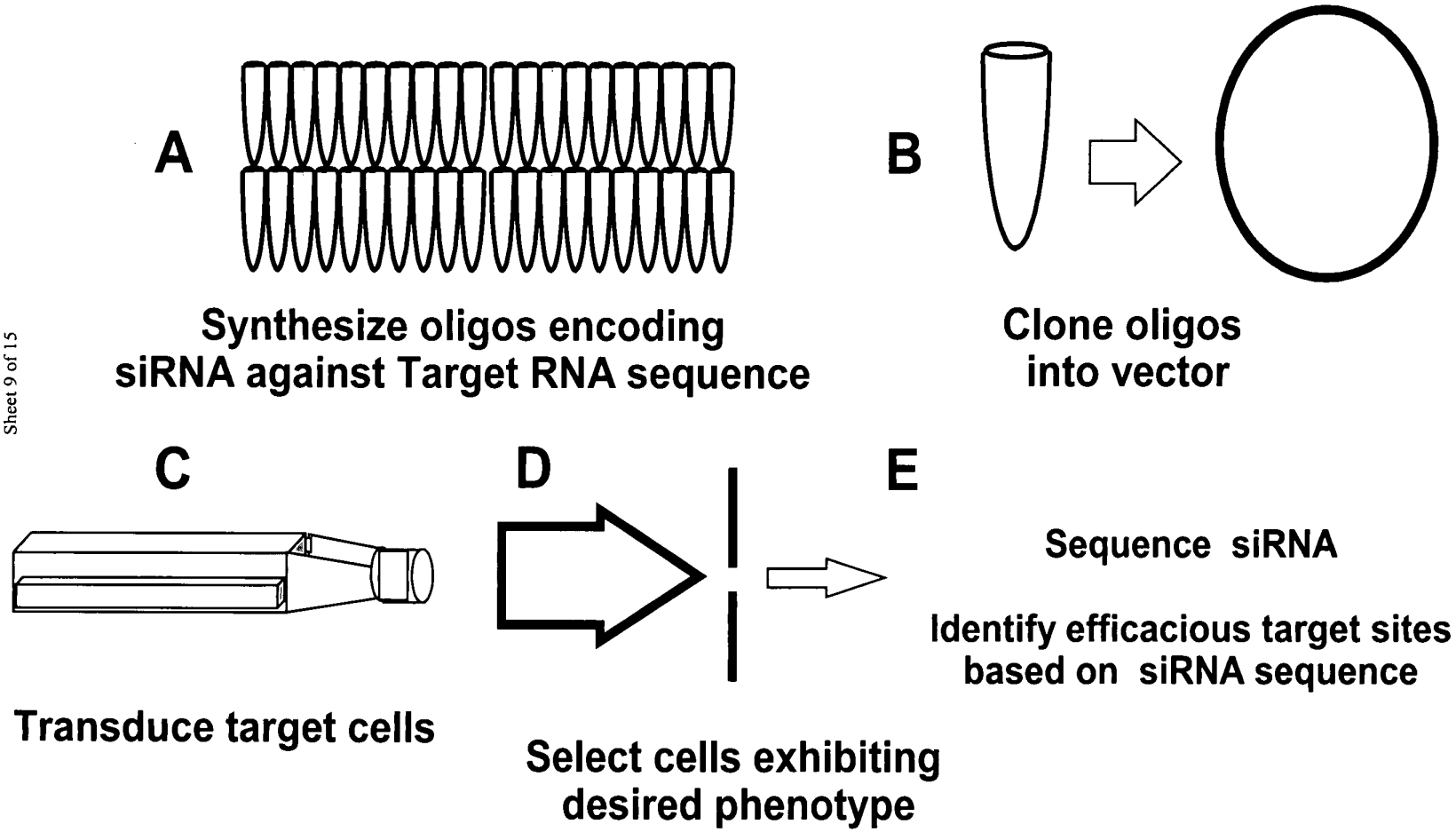
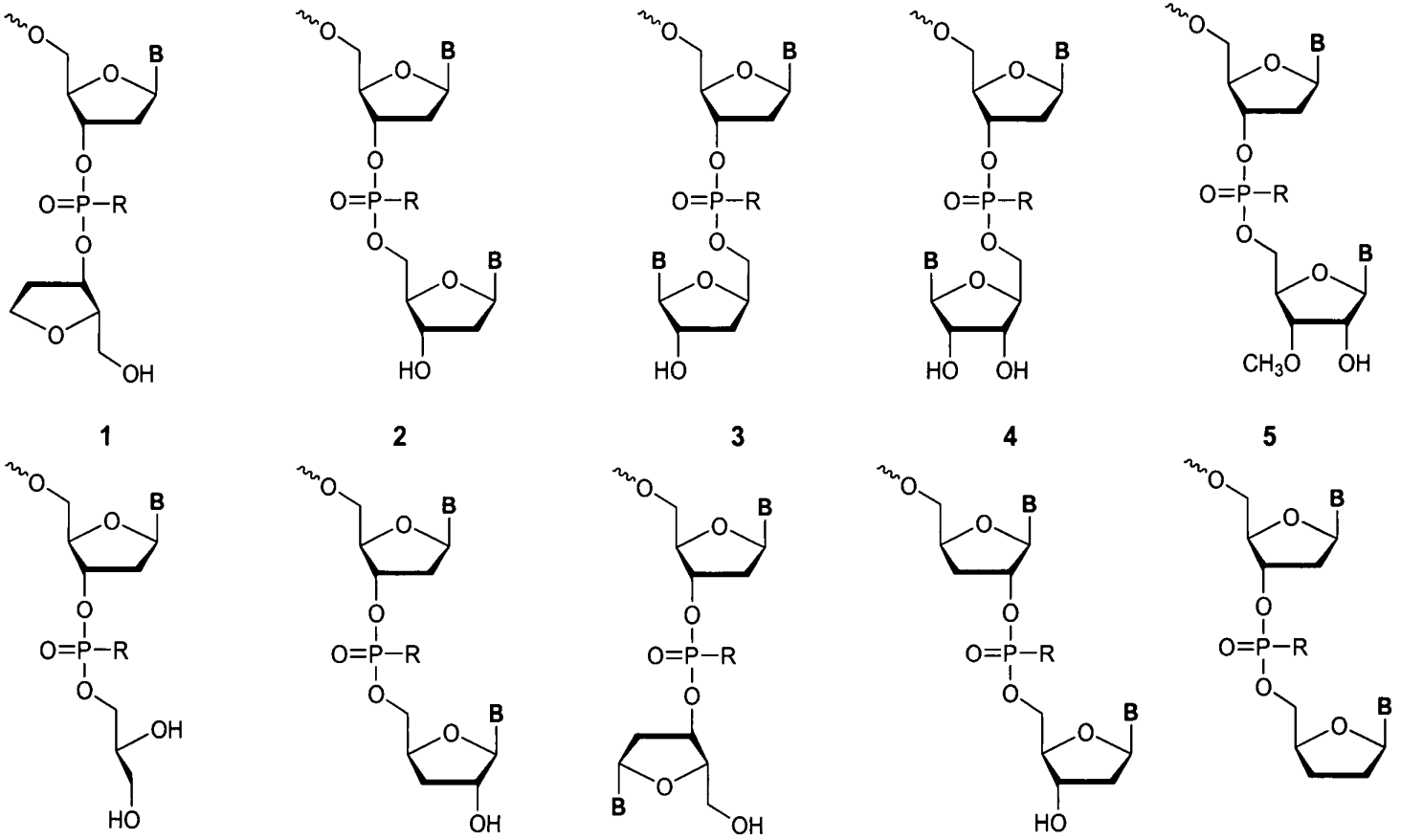


Figure 10



R = O, S, N, alkyl, substituted alkyl, O-alkyl, S-alkyl, alkaryl, or aralkyl
B = Independently any nucleotide base, either naturally occurring or chemically modified, or optionally H (abasic).

Figure 11: Modification Strategy

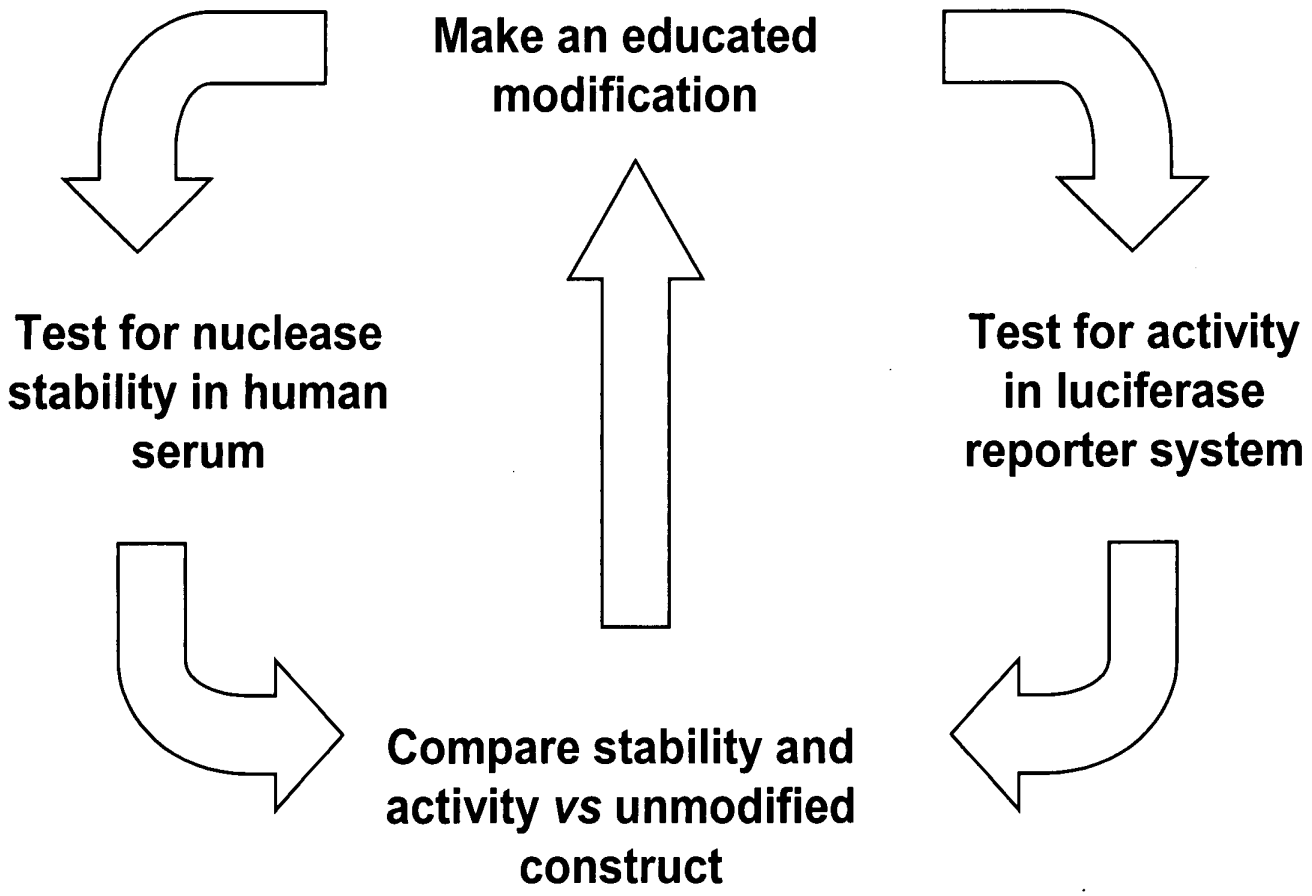
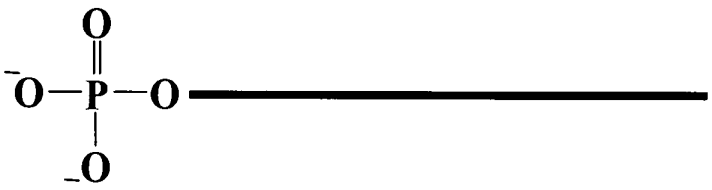
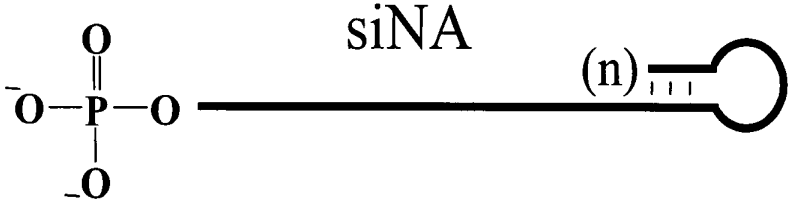


Figure 12: Phosphorylated siNA constructs

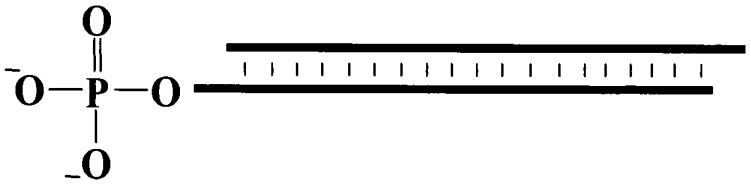


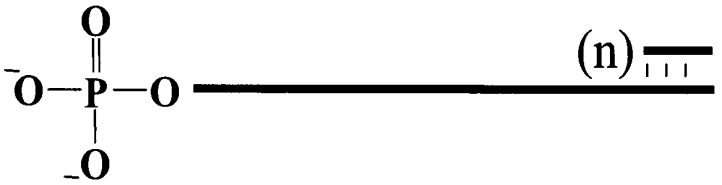
Phosphates can be modified as described herein

Asymmetric hairpin siNA



Asymmetric duplex siNA





(n) = number of base pairs (e.g. 3-18 bp)

Figure 13: 5'-phosphate modifications

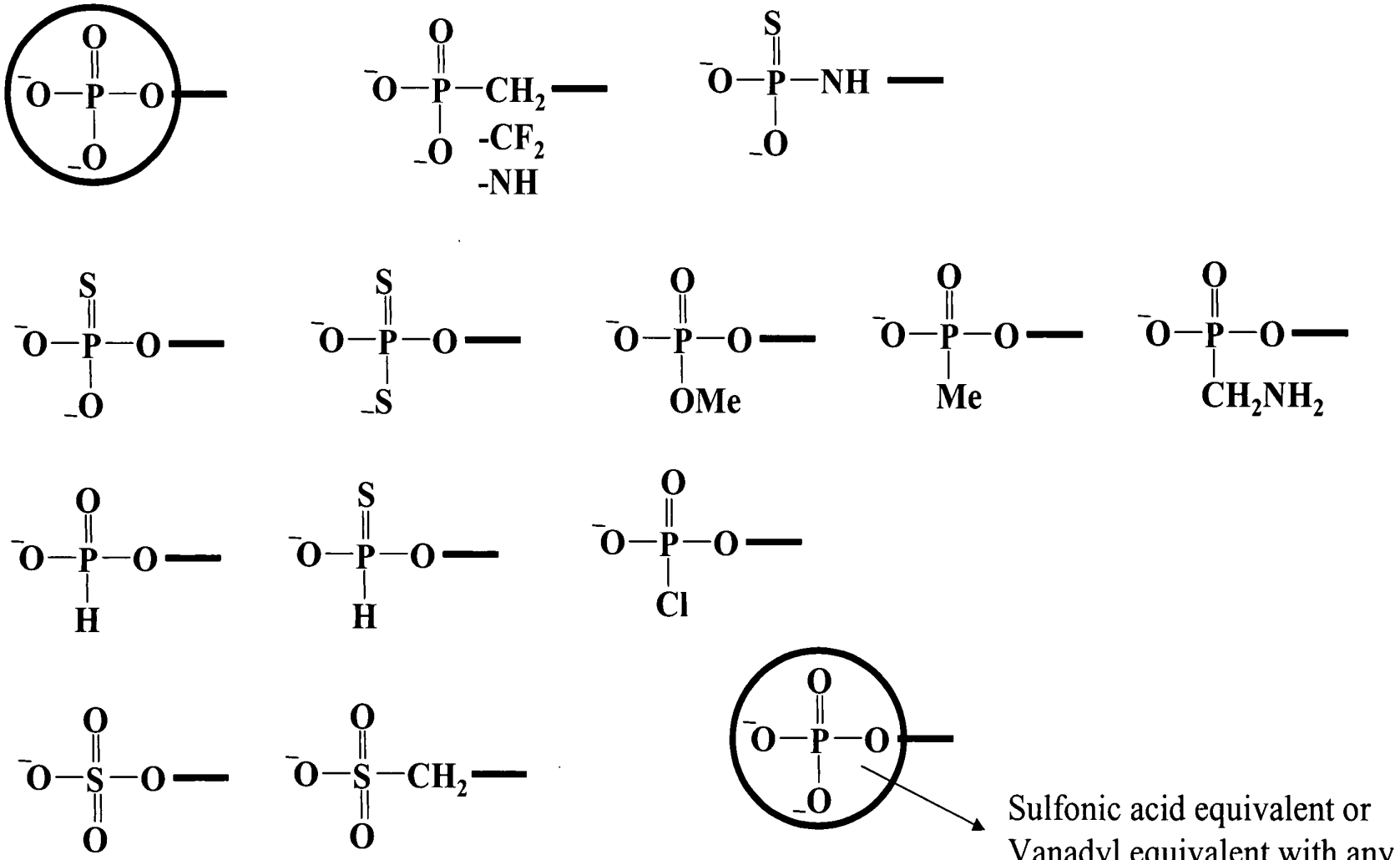


Figure 14A: Inhibition of VEGF RNA expression using Stab 0/0 and Stab 9/10 siNA targeting VEGF RNA sequences

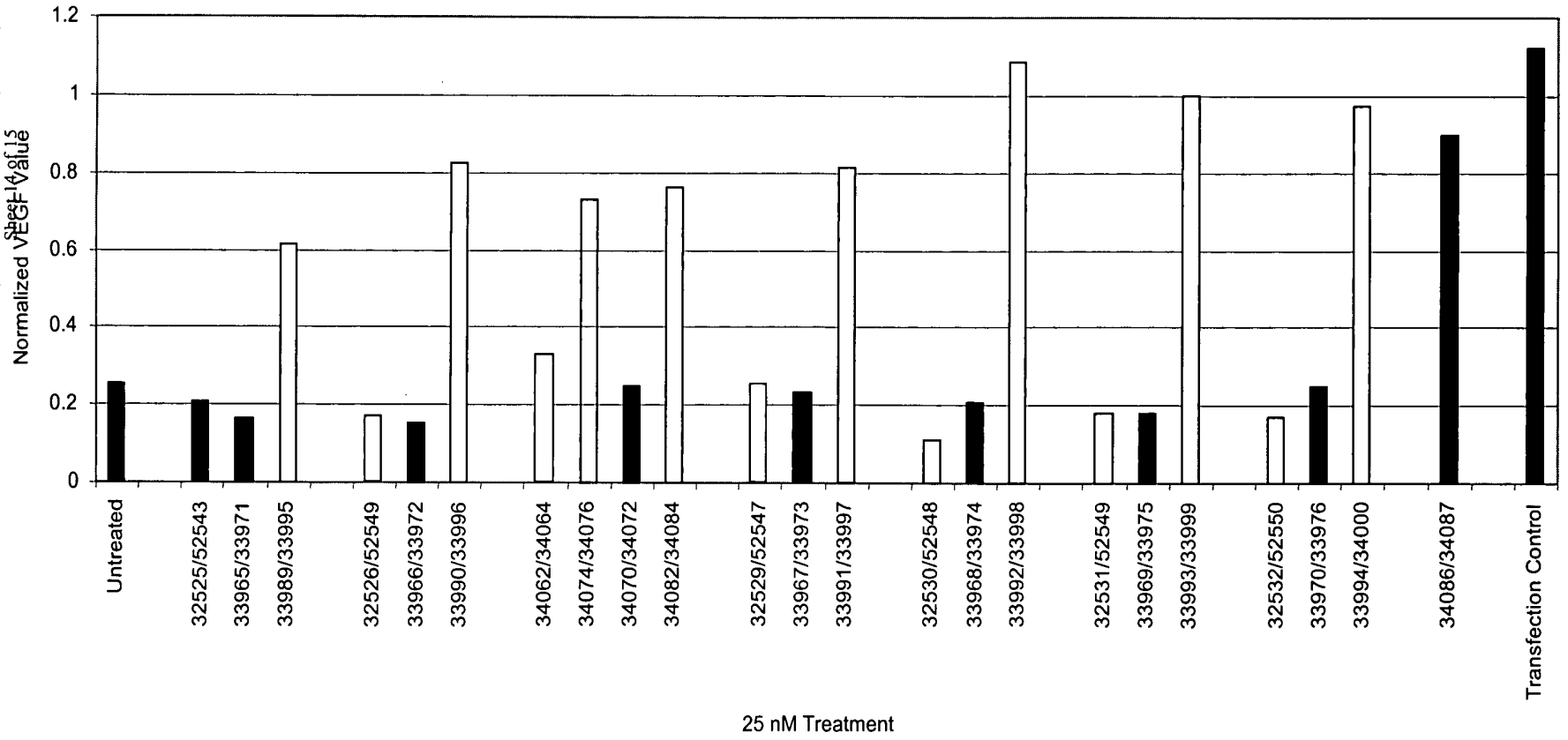


Figure 14B: Inhibition of VEGF RNA expression using Stab 7/8 siNA targeting VEGF RNA sequences